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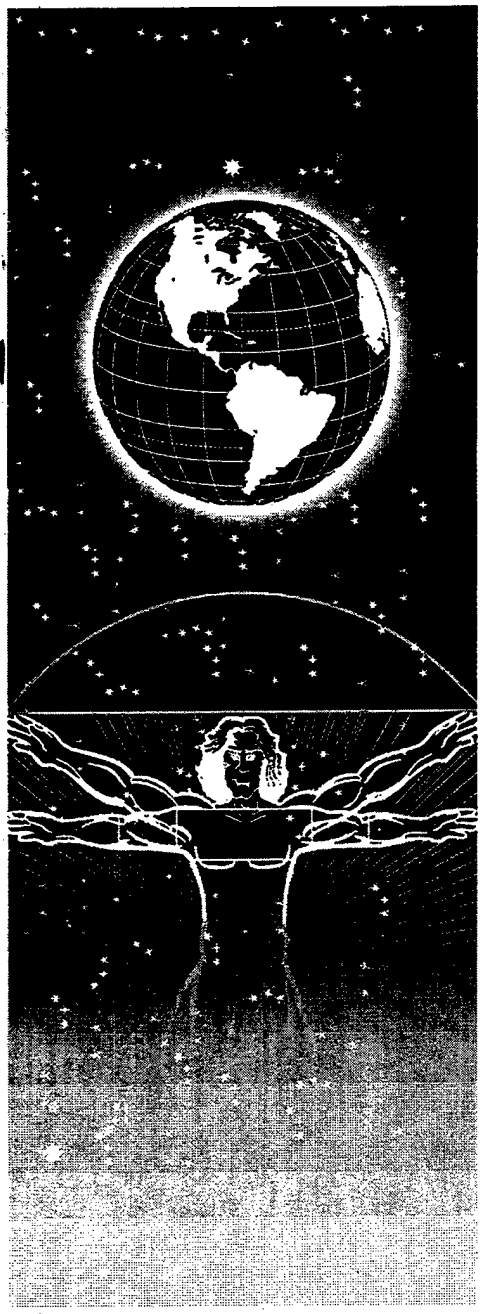

UNITED STATES AIR FORCE
ARMSTRONG LABORATORY

REPRODUCTIVE TOXICITY SCREEN OF TRIFLUOROIODOMETHANE (CF3I) IN SPRAGUE-DAWLEY RATS

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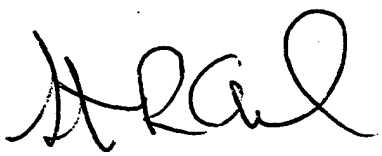
The experiments reported herein were conducted according to the "*Guide for the Care and Use of Laboratory Animals,*" Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



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CF_3I is being considered by the U.S. Air Force as a replacement for halon 1301 for fire-extinguishing requirements in unoccupied spaces. The purpose of this study was to determine and evaluate the potential for CF3I to produce reproductive toxicity and to provide additional information on the effect of CF3I exposure on the thyroid. Groups of 16 male and 16 female rats were exposed (6hr/day) to CF3I vapor at concentrations of 0 (control), 0.2, 0.7, and 2.0% using whole body inhalation chambers. Prior to mating, rats were exposed to CF3I for 4 weeks (5 days/wk). Exposures were 7 days/wk during the periods of mating (2 wk), gestation (3 wk), and lactation (3 wk). First generation pups were not exposed to CF3I vapor. In parental animals, there were no clinical signs of toxicity except for a minimal decrease in mean body weight in female rats at 2.0% CF3I. At necropsy, gross findings, mean serum chemistry levels, mean hematology values, mean bone marrow micronuclei scores, and mean organ weights were similar for all exposure groups, including the air control group. Statistically significant differences were considered incidental. There were no treatment-related histopathologic tissue findings, including the thyroid organ. Analysis of reproductive indices and parameters indicate CF3I is not a reproductive toxicant Results of serum thyroid hormone levels (e.g., T_3, T_4, rT_3, and TSH), indicated concentration-related increases in TSH, T_4, and rT_3. T_3 levels were decreased. First generation pup survival and mean body weights were similar in all exposure groups, including the control. Exposure of 2.0% CF3I vapor for approximately 14 weeks produced minimal general toxicity and no reproductive toxicity in Sprague-Dawley rats. On the basis of serum TSH concentrations, the no-observable-effect-level (NOEL) is 0.7% CF_3I.

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TABLE OF CONTENTS

LIST OF TABLES

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxicology Division under the ManTech Geo-Centers Joint Venture contract. This document serves as a final report on the reproductive toxicity screen of the halon replacement candidate trifloroiodomethane. The research described in this report began in December 1996 and was completed in January 1998 under Department of the Air Force Contract No. F41624-96-C-9010. Lt Col Terry A. Childress served as the Contracting Officer's Representative for the U.S. Air Force, Armstrong Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for ManTech Geo-Centers Joint Venture.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended. The authors gratefully acknowledge the excellent technical assistance of Richard J. Godfrey, Willie Malcomb, Gerry Buttler, Jerry W. Nicholson, and Margaret A. Parish of ManTech Environmental, Wright-Patterson AFB, OH; and Drs. Sheela Sharma and Luther Smith and their staffs of ManTech Environmental, Research Triangle Park, NC.

ABBREVIATIONS

AFRL/HEST	Air Force Research Laboratory/Crew Survivability & Logistics Operational Toxicology
ALB/GLOB	albumin/globulin
ALKP	alkaline phosphatase
ALT	alanine aminotransaminase
ANOVA	analysis of variance
AST	aspartate aminotransaminase
BUN	blood urea nitrogen
°C	degrees Celsius
CF_3I	trifluoroiodomethane
d	day(s)
EPA	Environmental Protection Agency
°F	degrees Fahrenheit
g	gram(s)
GC/MS	gas chromatography/mass spectrometry
gd	gestation day
HGB	hemoglobin
HCT	hematocrit
hr	hour(s)
L	liter(s)
ld	lactation day
m	meter(s)
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
mg	milligram(s)
min	minute(s)
mm Hg	millimeters mercury
MN	micronuclei
µm	micrometer(s)
n	size of group or set
NOAEL	no observable adverse effect level
NOEL	no observable effect level
PCE/NCE	polychromatic erythrocytes/normochromatic erythrocytes
PLT	platelet(s)
ppm	parts per million
RBC	red blood cell(s)
RIA	radioimmunoassay
rT_3	reverse triiodothyronine
SD	standard deviation
SD rat	Sprague-Dawley rat
T_3	triiodothyronine
T_4	thyroxine
TSH	thyroid-stimulating hormone
v/v	volume per volume
WBC	white blood cell(s)
wk	week(s)

SECTION I

INTRODUCTION

Environmental concern over the depletion of stratospheric ozone and global warming has led to an international treaty called the The Montreal Protocol which calls for the phase out of halons by the year 2000. Presently, the U.S. Air Force is using Halon 1301 (CF_3Br) as a flooding agent for extinguishing in-flight aircraft and electronic equipment fires and for fire extinguishment in confined spaces. Because it has less ozone depleting activity and excellent fire suppression properties, trifluoroiodomethane (CF_3I) is being considered as a replacement of Halon 1301 for fire extinguishing requirements in unoccupied spaces. No information is available in the literature concerning the potential for CF_3I to produce reproductive toxicity. The purpose of this study was to determine and evaluate the potential for CF_3I to produce reproductive toxicity in the rat. Previous toxicity studies in the rat indicated that the thyroid and bone marrow were "target" organs, i.e., organs sensitive to CF_3I exposure. This study also determined the effect of CF_3I exposure on the thyroid and bone marrow.

Background

CF_3I has a high vapor pressure under ambient conditions (541 mm Hg at 25°C), thus inhalation is a major route of exposure for persons in the workplace. Some information is available in the literature concerning CF_3I toxicity. A modified acute inhalation toxicity test was performed in which rats were exposed in a nose-only chamber to 12% CF_3I for 15 min (Ledbetter,1993). Excess salivation was observed in the rats upon removal from the chamber; however, all rats appeared to be fully recovered by 2 hr postexposure. A 15-min, nose-only inhalation study in rats determined the LC_{50} value to be 27% CF_3I (Ledbetter, 1994). As part of the process to develop environmental and health effects criteria, acute, 2-wk, and 13-wk nose-only inhalation toxicity studies were conducted in Fischer 344 rats (Dodd *et al.*, 1997a). In the acute study, rats were exposed to 1.0, 0.5 or 0 (control) % CF_3I for 4 hr and sacrificed immediately following exposure, 3 d postexposure or 14 d postexposure. There were no deaths and no clinical signs of toxicity throughout the study. Histopathologic examination of select tissues showed no lesions of pathologic significance. In the 2-wk study, rats were exposed for 2 hr/d, 5 d/wk to 12, 6, 3 or 0% CF_3I. No deaths were observed, though lethargy and slight incoordination were noted in rats of the 12 and 6% groups at the conclusion of each daily exposure. Mean body weight gains were depressed in rats of the 12% and 6% groups. Serum reverse T_3 (rT_3) values were increased at all exposure levels. At necropsy, no gross lesions or differences in absolute or relative organ weights were noted. Histopathologic examination of the thyroid and parathyroids indicated no morphological abnormalities in the CF_3I-exposed rats.

In the 13-wk study, four groups of 15 male and 15 female rats were exposed to 8, 4, 2 or 0% CF_3I 2 hr/d, 5 d/wk for 13 wk. Rats exposed to 8 or 4% CF_3I had lower mean body weights than the controls. Deaths observed in the 2 and 8% groups were attributed to accidents resulting from the restraint system employed. Hematologic alterations were minimal and considered insignificant. Increases in the frequency of micronucleated bone marrow polychromatic

erythrocytes were observed in rats of all three CF_3I groups. Serum chemistry alterations observed in rats of all CF_3I exposure groups included decreases in T_3 and increases in rT_3, T_4, and TSH. Relative organ weight increases (8% CF_3I group) occurred in the brain, liver and thyroid; decreases were observed in the thymus and testes. A decrease in relative thymus weights and an increase in relative thyroid weights were observed also in rats of the 2 and 4% groups. Histopathological findings included a mild inflammation in the nasal turbinates of rats exposed to 4 or 8% CF_3I, mild atrophy and degeneration of the testes (4 and 8% CF_3I groups), and a mild increase in thyroid follicular colloid content in rats of all CF_3I exposure groups.

In genotoxicity testing protocols (Dodd *et al.*, 1997b) CF_3I was positive with and without metabolic activation in the *Salmonella typhimurium* histidine reversion mutagenesis assay. The L5178Y/*tk* mouse lymphoma cell mutagenesis assay showed that CF_3I did not induce gene or chromosomal mutations in mammalian cells *in vitro*. However, a positive evaluation in the mouse bone marrow erythrocyte micronucleus test indicated that CF_3I was clastogenic *in vivo*. Cardiac sensitization testing of CF_3I vapor using beagle dogs showed a no observable effect level at 0.2% and a lowest observable adverse effect level at 0.4% (Dodd and Vinegar, 1998). This toxicity precludes the use of this compound as a flooding agent in occupied spaces, but it may still be used in unoccupied spaces (Vinegar *et al.*, 1995). Gas uptake kinetics of CF_3I have been studied (Williams *et al.*, 1994), and a physiologically-based pharmacokinetic model has been developed to simulate blood concentrations of CF_3I during inhalation exposures (Vinegar and Jepson, 1996).

Results from the repeated exposure mammalian studies and the genotoxicity tests suggest that additional studies are needed to complete a toxicity profile with CF_3I. No reproductive or developmental toxicity data are available. Further, a no observable adverse effect level (NOAEL) has not been established for CF_3I in the rat.

Study Objective

The purpose of this investigation was to determine and evaluate the potential of CF_3I to produce alterations in parental fertility; maternal pregnancy and lactation; and growth and development of offspring in the rat. A no-observable-adverse-effect-level (NOAEL) for CF_3I was to be determined. Additionally, efforts were made to answer the following questions posed by recently completed toxicity studies on CF_3I. Are the testicular effects that were observed in rats in the 13-week study transient, an artifact, or a reproductive hazard? Are the genotoxic effects that were observed in both *in vitro* and *in vivo* tests persistent at low exposure concentrations, and do they pose a genetic hazard? Are the thyroid effects that were observed in rodent subchronic studies persistent at low exposure concentrations, and do they pose a functional hormonal hazard? Exposure concentrations selected for this study extended beyond those of the 13-wk study and all target organs were thoroughly examined.

SECTION II

MATERIALS AND METHODS

Test Material

The test material, trifluoroiodomethane (CAS no. 2314-97-8), has a boiling point of -22.5 °C and a vapor pressure of 541 mm Hg @ 25 °C. The supplier of CF_3I test material was Ajay North America, LLC., Powder Springs, GA. The purity of the test material was determined by gas chromatography/mass spectrometry (GC/MS). The test material was analyzed for the presence of fluoride ion using a Combination Fluoride Ion Electrode, since decomposition products of CF_3I are likely to include hydrogen fluoride and hydrogen iodide. More details are given in Appendix A.

Laboratory Animals and Animal Husbandry

The Sprague-Dawley (SD) albino rat is the species of choice for reproduction studies because of high fecundity (EPA Health Effects Testing Guidelines, 40 CFR, section 798.4700). AFRL/HEST and contractor personnel have conducted several reproductive toxicity screens using the SD rat. Thus, in-house historical control data were available to help interpret study results. Seventy (70) male and sixty-seven (67) female (nulliparous and non-pregnant) Sprague-Dawley Crl:CD®(SD)BR rats, 8-9 weeks of age, were ordered and received from Charles River Breeding Laboratories, Hollister, CA.

Routine animal husbandry procedures were performed by AFRL/HEST personnel using Standard Operating Procedures for rodents. The animals to be used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996, and the Animal Welfare Act of 1966, as amended.

The rats were housed in wire bottom cages during exposure (one/cage except during the mating period) and in plastic shoe-box cages with bedding (two males/cage, one female/cage, except during the mating period) during non-exposure periods. During the exposure periods, rats were assigned to specific cage locations. The exposure cages were rotated in a clockwise manner within the chamber each exposure day. Appropriate bedding and care (minimal stress, such as noise) were required for the dams during their gestation period and for the dams and their pups during the lactation interval. A 12-hr light/dark cycle was provided. Temperature was maintained between 21 and 26°C, and relative humidity was maintained between 35 and 65%. Food (certified Purina Formulab) and water were available *ad libitum* during nonexposure periods. Veterinary care was provided by the AFRL/HEST veterinary staff while the animals were in animal rooms and by study investigators during exposure periods and critical periods of gestation and lactation.

The animals were housed in Building 838 upon receipt for quarantine and quality control procedures. Inhalation exposures occurred in Building 79, Room 153. During nonexposure periods, rats were housed in laminar-flow rooms in Building 79.

Experimental Design

The study had 16 male and 16 female rats/group to yield at least 12 pregnant females at term. Animals were assigned to the different groups by means of a computer-generated randomization stratified by body weight such that the body weight means of all groups were homogeneous by statistical analysis at exposure initiation.

Group Assignments and Exposure Levels:

Group	No. Animals		Exposure Concentration (%)
	Male	Female	
Control	16	16	0.0
Low	16	16	0.2
Middle	16	16	0.7
High	16	16	2.0
Positive control*	6	3	no exposure

*These rats were used only to serve as positive controls for the micronuclei in bone marrow erythrocytes assay. There were no chamber exposure for these rats; they remained in home (plastic shoebox) cages.

The animals were exposed to CF_3I vapor for four weeks (6 hr/day, 5 days/week) prior to mating. The animals were mated for 14 days within their appropriate treatment level. During the mating, gestation and lactation phase, animals were exposed 6 hr/day, 7 days/week. However, dams were not exposed from Gestation Day 21 through Lactation Day 4 to allow for parturition and early lactation. Pups were not placed in exposure chambers, but remained in home cages separated from the dams for 6 hr/day during Lactation Days 5 through 21. Following gestation Day 21 of the last female on study to deliver pups, exposure to CF_3I vapor (or air) returned to 6 hr/day, 5 days/week, until the final day of termination for all remaining animals on study.

General Procedures and Experimental Evaluations

Test Material Generation and Analysis

Whole-body inhalation exposures were performed in 690-L chambers made of stainless steel and glass, similar in design to those described by Hinners *et al.*, *Arch Environ Health* 16, 194-206, 1968. The CF_3I and air for dilution were controlled through flow meters. Fine control of chamber concentration was made by minor adjustment of the CF_3I flow in response to chemical analysis of the chamber atmosphere. Total chamber air flow was approximately 60L/min. Relative humidity and temperature of the exposure atmosphere were constantly monitored and recorded. Continuous analysis of the chamber air for CF_3I was performed using infrared absorption spectrometers. Instrumental calibration was performed using known concentrations

of freshly prepared CF_3I in air contained in tedlar sample bags. Calibration checks were performed at appropriate intervals. Chamber atmosphere analyses of CF_3I were expressed in per cent by volume or ppm (v/v). More details are given in Appendix A.

Parental Animals - Clinical Observations and Body Weights

The animals were observed twice daily (a.m. and p.m.), including weekends and holidays. Signs of toxicity were recorded. The body weight of the male rats were determined prior to the first exposure and weekly thereafter. The body weights of female rats were determined and recorded in the same manner until confirmation of mating. During gestation, female rats were weighed on Gestational Days 0, 7, 14 and 20. Dams producing litters were weighed on Days 0, 4, 7, 12 and 21 postpartum.

Parental Animals - Mating Procedures

Animals of the parental generation were approximately 11 weeks of age at the commencement of exposure. They were exposed for four weeks prior to mating. The animals were then mated on the basis of one male to one female selected randomly within each exposure group for a maximum time period of 14 days. There was no replacement of males if mating did not occur. The observation of vaginal or dropped copulatory plugs and /or vaginal sperm were considered evidence of successful mating. Females were examined twice daily (a.m. and p.m.) during the cohabitation period for dropped copulation plugs. Any female not exhibiting a dropped copulation plug was examined for the presence of a copulation plug or sperm in the vaginal tract once daily (a.m.). The day a copulation plug or vaginal sperm was observed was designated Gestation Day (gd) 0. Once a plug or vaginal sperm was observed, the mated pair were individually housed. For any female that did not show evidence of successful mating after 14 days of cohabitation, the last scheduled mating day was considered gd 0 for that female and the animal was treated similarly to the other dams for subsequent events. Females were observed twice daily beginning on gd 20 for evidence of littering. The dams were allowed to rear their young to day 21 postpartum. Standardization of litter size was performed on lactation day 4.

Parental Animals - Clinical Pathology

Routine hematology and clinical chemistry evaluations were conducted on blood samples taken immediately prior to termination from all animals. The blood was sampled via the posterior vena cava. Hematology and serum chemistry assays included red blood cell count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hematocrit, total and differential leukocyte count, platelet count, total protein, albumin, globulin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase, urea nitrogen, creatinine, calcium, glucose, potassium, phosphorus, sodium, magnesium, trigylcerides and cholesterol. Hematologic and serum chemistry parameters were determined according to established procedures, utilizing a Cell-Dyn 3500 (Abbott Diagnostics, Chicago, IL) and a Vitros 250XR (Johnson and Johnson, Rochester, NY), respectively.

The following serum thyroid hormone levels were determined in control and CF_3I exposed rats: thyroxine (T4), triiodothyronine (T_3), reverse T_3 (rT_3), and thyroid-stimulating hormone (TSH). Assays for T_4, T_3, rT_3, and TSH were performed using radioimmunoassay (RIA) kits and were carried out according to manufacturer's instructions. For all thyroid hormone or TSH measurements, assay kits were prepared for each animal termination period (study week 7 or 14) with the same batch number and the same expiration date. Tracer (^{125}I) radioactivity was measured with a Packard gamma counter (Packard Instrument Co., Meriden, CT). For T_3, the RIA assay kit was purchased from Diagnostic Product Corp. (Los Angeles, CA), and canine T_3 antibody coated tubes were used. For T_4, the RIA assay kit was purchased from Diagnostic Product Corp. (Los Angeles, CA), and T_4 antibody coated tubes were used. For rT_3, the RIA assay kit was purchased from Wein Laboratories (Succasunna, NJ), and rT_3 antiserum raised in the rabbit was used. For TSH, the RIA assay kit was purchased from Amersham Corp. (Arlington Heights, IL), and both lyophilized rabbit anti-rat TSH serum and Amerlex-M second antibody (donkey anti-rabbit serum coated onto magnetized polymer particles containing sodium azide) were used.

Parental Animals - Micronuclei in Bone Marrow Erythrocytes

Bone marrow cells were collected from the femur and smears were prepared from all rats. Positive control rats were administered a single dose of cyclophosphamide (7.5 mg/kg) intraperitoneally 24 hrs prior to termination. Slides were stained by the Giemsa/May-Greenwald method and observed microscopically at 100×. The frequency of micronucleated cells were evaluated by random observation of 1000 polychromatic erythrocytes (PCE) per sample. The ratio between PCE and normochromatic erythrocytes (NCE) was determined by scoring approximately 1000 erythrocytes as an indicator of toxicity of the test agent.

Parental Animals - Termination, Gross Necropsy, Organ Weights, and Histopathology

Following the 14-day mating period, eight (8) male rats/group were terminated (Study Week 7). The remaining 8 male rats/group and 16 female rats/group were terminated over a 5-day period subsequent to when the last female rat on study reached lactation Day 21. Exposure to CF_3I continued on a daily basis until the final day of termination. (Note: positive control rats for micronuclei determination were terminated at each of these two termination periods.)

Animals were not fasted prior to termination, since fasting might affect thyroid hormone levels. The gross examination at necropsy included external surfaces, all body orifices, the thoracic, abdominal, and pelvic cavities and their viscera, cervical tissues, implantation sites, organs, and brain.

Organs weighed included adrenal glands, heart, lungs, liver, kidneys, ovaries, testes, epididymides, brain, spleen, and thymus. Thyroid glands (including parathyroid glands) were removed with the trachea, fixed in buffered formalin, then dissected free of the trachea, and weighed.

The following tissues were removed from all animals and preserved for possible histopathological examination. Select tissues (underscored below) from male and female rats of the control and high-dose groups were subjected to histopathologic examination. Target organs

only, identified during examination of the high-dose and control groups, were to be examined from animals at the mid- and low-dose groups.

-liver
-kidneys
-brain
-pituitary gland
-vagina
-uterus
-ovaries, including corpora lutea
-spleen
-stomach
-duodenum
-adrenal glands
-thyroid glands
-parathyroid glands
-other tissues with gross lesions identified as being potentially treatment related
-testes*
-epididymides*
-scrotum
-seminal vesicles
-prostate glands
-bone marrow (sternal and femoral) sections
-bone marrow smear
-colon
-ileum
-urinary bladder
-heart
-pancreas
-thymus
-complete respiratory tract (nasal cavity, trachea, bronchi, lungs)

*Fixed in Bouin's Fixative; sections stained with periodic acid and Schiff's (PAS); counterstained with hematoxylin.

For animals dying during the study, a complete gross necropsy and histopathologic examination were conducted to determine the possible cause of death.

Progeny

All pups were examined as soon as possible after birth to determine number of viable and stillborn members of each litter. Survival indices were calculated at 0, 4, 7, 14, and 21 days after birth. Live pups were counted, sexed, and examined grossly at birth (postnatal day 0) and weighed individually on days 1, 4, 7, 14, and 21 after birth. Standardization of litter sizes, 4 per sex selected randomly when possible, occurred on postnatal day 4. Pups were examined for physical abnormalities at birth and observed daily throughout the postpartum period. Pups dying during this period were necropsied when possible to investigate the cause of death. Pups were terminated at weaning, postnatal Day 21, and subjected to a gross pathologic examination.

Statistical Analysis

For body weight data, a repeated measures analysis of variance (ANOVA) was conducted. An ANOVA with Bonferroni multiple comparisons was conducted on the thyroid hormone data. Fisher's Exact test was used on the reproductive data that are calculated on a group basis (e.g., gestational index). For reproductive data calculated on an individual basis (e.g., live birth index), micronuclei data, hematology and serum chemistry data, and organ weight data, the Wilcoxon Rank Sum test was used to increase the statistical sensitivity when comparing control group values to treated group values.

SECTION III

RESULTS

Test Material Analysis, Chamber Atmosphere Analysis and Chamber Environment

Details of test material and chamber atmosphere analyses are given in Appendix A. The test material was 99.7+% CF_3I and remained stable throughout the study period. Results of the daily analytical and nominal mean concentrations of CF_3I, chamber temperature, and chamber relative humidity for each exposure group are given in Table 1. Analytical chamber concentrations of CF_3I matched the target exposure concentrations of 0 (control), 0.2, 0.7, and 2.0%. Chamber temperature and relative humidity means for all exposure groups ranged from 73.5 to 74.2°F and 43.4 to 54.8%, respectively.

TABLE 1. CHAMBER ATMOSPHERE ANALYSIS OF CF_3I AND CHAMBER ENVIRONMENT[a]

Target CF_3I Concentration (%)	Analyzed CF_3I Concentration (%)	Nominal CF_3I Concentration (%)	Analytical to Nominal Ratio	Chamber Temperature (°F)	Chamber Relative Humidity (%)
0	0.0	0.0		74.2 ± 1.6	54.8 ± 6.5
0.2	0.20 ± 0.01	0.24 ± 0.01	0.83	73.9 ± 1.8	43.4 ± 5.2
0.7	0.71 ± 0.01	0.72 ± 0.03	0.99	74.1 ± 1.7	50.5 ± 5.0
2.0	2.02 ± 0.03	2.08 ± 0.09	0.97	73.5 ± 1.6	54.8 ± 6.5

[a]Values are reported as mean ± SD of daily means where appropriate

Parental Animals

Clinical Observations and Body Weights

Rats were observed at least twice-a-day (morning and afternoon), including inhalation exposure periods. There were no treatment-related clinical findings. Areas of alopecia were sporadic and considered incidental. Mean body weights are given in Tables 2 (male rats) and 3 (female rats).

TABLE 2. BODY WEIGHTS[a] OF MALE RATS EXPOSED TO CF_3I

Study Day	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
0	346 ± 16 (16)	347 ± 17 (16)	342 ± 18 (16)	342 ± 24 (16)
7	385 ± 18 (16)	385 ± 26 (16)	383 ± 20 (16)	377 ± 30 (16)
14	418 ± 20 (16)	416 ± 31 (16)	413 ± 25 (16)	405 ± 35 (16)
21	445 ± 24 (16)	442 ± 36 (16)	442 ± 28 (16)	432 ± 41 (16)
28	465 ± 24 (16)	456 ± 39 (16)	462 ± 34 (16)	443 ± 40 (16)
35	485 ± 22 (16)	480 ± 40 (16)	486 ± 39 (16)	465 ± 48 (16)
42	507 ± 22 (16)	504 ± 40 (16)	511 ± 38 (16)	488 ± 50 (16)
49	521 ± 23 (8)	530 ± 55 (8)	508 ± 61 (8)	507 ± 59 (8)
56	534 ± 26 (8)	539 ± 58 (8)	524 ± 51 (8)	518 ± 64 (8)
63	549 ± 27 (8)	558 ± 61 (8)	546 ± 53 (8)	531 ± 65 (8)
70	565 ± 27 (8)	571 ± 66 (8)	552 ± 40 (8)	536 ± 65 (8)
77	580 ± 31 (8)	580 ± 74 (8)	566 ± 48 (8)	548 ± 70 (8)
84	581 ± 32 (8)	591 ± 78 (8)	577 ± 51 (8)	558 ± 74 (8)
91-92[b]	595 ± 26 (8)	599 ± 79 (8)	586 ± 51 (8)	567 ± 74 (8)

[a]Mean ± SD, grams, (n)
[b]day of euthanasia

TABLE 3. BODY WEIGHTS[a] OF FEMALE RATS EXPOSED TO CF_3I

Day[b]	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
SD 0	224 ± 14 (16)	228 ± 13 (16)	224 ± 15 (16)	228 ± 17 (16)
SD 7	241 ± 15 (16)	237 ± 17 (16)	235 ± 9 (16)	231 ± 19 (16)
SD 14	250 ± 16 (16)	248 ± 20 (16)	246 ± 15 (16)	238 ± 21 (16)
SD 21	264 ± 18 (16)	259 ± 20 (16)	259 ± 14 (16)	249 ± 24 (16)
GD 0	269 ± 18 (15)	269 ± 21 (12)	268 ± 18 (15)	265 ± 24 (11)
GD 7	299 ± 20 (15)	302 ± 23 (12)	294 ± 16 (15)	289 ± 22 (11)
GD 14	326 ± 21 (15)	336 ± 23 (12)	330 ± 17 (15)	323 ± 23 (11)
GD 20	396 ± 26 (15)	407 ± 37 (12)	401 ± 23 (15)	389 ± 32 (11)
LD 0	308 ± 26 (15)	314 ± 12 (12)	310 ± 21 (15)	311 ± 21.6 (11)
LD 4	325 ± 22 (15)	327 ± 17 (12)	322 ± 15 (15)	320 ± 23 (10)
LD 7	327 ± 20 (15)	327 ± 20 (12)	322 ± 12 (15)	318 ± 25 (10)
LD 14	319 ± 37 (15)	337 ± 21 (11)	332 ± 24 (15)	331 ± 20 (10)
LD 21	334 ± 16 (15)	334 ± 17 (11)	329 ± 16 (15)	326 ± 22 (10)
SD 93-95[c]	321 ± 27 (16)	322 ± 25 (15)	314 ± 19 (16)	289[d] ± 36 (16)

[a]Mean ±SD, grams, (n)
[b]SD = Study Day; GD = Gestational Day; LD = Lactational Day
[c]day of euthanasia
[d]$p<0.01$ compared to control

Clinical Observations and Body Weights - cont'd

Though not statistically significant, except for the final body weight values in female rats (Day 93-95), there was a marginal decrease in mean body weights of the 2.0% group compared to the control group. The mild decrease in absolute body weights for this group of rats is due primarily to a depression in body weight gain during the first 2-3 weeks of the study. Body weight values were normal for male and female rats of the 0.2 and 0.7% CF_3I groups.

Clinical Pathology - Hematology and Serum Chemistry

Hematology and serum chemistry values (except thyroid hormones) for male rats are given in Tables 4 through 7. Similar information is given for the female rats in Tables 8 and 9. For male rats, statistical significant differences in hematology and serum chemistry parameters were few in number and not considered treatment related due to 1) lack of consistency with time (7-wk compared to 14-wk values), 2) lack of dose-response, or 3) the difference was of small magnitude and not considered biologically important. For female rats, statistically significant differences in hematology and serum chemistry parameters were not considered CF_3I exposure related for the same reasons stated above. Though mild increases in cholesterol, total protein, and albumin were observed in both the 0.7 and 2.0% CF_3I groups of female rats (Table 9), a concentration-related increase was lacking.

Clinical Pathology - Serum Thyroid Hormones

Serum thyroid hormone values for male rats after 7 wk exposure to CF_3I are given in Table 10. Table 11 has thyroid hormone levels for male and female rats after 14 wk exposure to CF_3I. Statistically significant and concentration-related increases in T_4 and rT_3 were observed in male and female rats after 7 or 14 wk of exposure. T_3 levels were decreased in a concentration-related manner in these animals. For TSH, an increase was observed in CF_3I-exposed male rats after 7 wk exposure, but there was no concentration relationship. After 14 weeks exposure, male and female rats of the 2.0% group had statistically significant increases in TSH levels compared to control rats. A marginal and statistically significant increase in TSH was observed in female rats of the 0.2% group, but this effect was not statistically significant in female rats at 0.7% CF_3I.

Micronuclei in Bone Marrow Erythrocytes

Micronuclei scores for male and female rats exposed to CF_3I for 7 or 14 wk were similar to micronuclei scores in the control animals (Table 12). Positive control animals had mean micronuceli scores that were two- to five-fold higher than negative control values (data not shown). The weak response in the positive control animals was due to the administration of a small dose of cyclophosphamide to induce micronuclei formation. The ratio of PCE/NCE (an indicator of bone marrow cell toxicity) was similar in all study groups, control or CF_3I exposure (Table 12).

TABLE 4. HEMATOLOGY VALUES[a] FOR MALE RATS EXPOSED TO CF_3I FOR 7 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=8	n=8	n=8	n=8
WBC ($x10^3/mm^3$)	8.9 ± 3.2	9.2 ± 1.9	8.7 ± 1.6	10.3 ± 3.9
Neutrophils (%)	12 ± 2	12 ± 3	12 ± 3	16[b] ± 4
Lymphocytes (%)	86 ± 2	85 ± 4	84 ± 4	81[b] ± 5
Monocytes (%)	1.0 ± 1.4	1.3 ± 1.6	2.4 ± 2.1	1.8 ± 2.3
Esoinophils (%)	1.6 ± 0.5	1.5 ± 0.5	1.2 ± 0.6	1.0[b] ± 0.3
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1
RBC ($x10^6/mm^3$)	8.34 ± 0.41	8.33 ± 0.48	8.31 ± 0.21	8.17 ± 0.47
HGB (g/dL)	14.9 ± 0.5	14.9 ± 0.5	14.6 ± 0.4	14.5 ± 0.7
HCT (%)	48 ± 2	47 ± 2	47 ± 1	46 ± 2
MCV (fL)	57 ± 1	57 ± 2	57 ± 2	57 ± 2
MCH (pg)	18 ± <1	18 ± 1	18 ± <1	18 ± 1
MCHC (g/dL)	31 ± <1	32 ± 1	31 ± <1	31 ± 1
PLT ($x10^3/mm^3$)	1164 ± 130	1140 ± 164	1120 ± 158	1092 ± 81

[a]Mean ± SD
[b]$p<0.05$ compared to control

TABLE 5. SERUM CHEMISTRY VALUES[a] FOR MALE RATS EXPOSED TO CF_3I FOR 7 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=8	n=8	n=8	n=8
Glucose (mg/dL)	222 ± 27	225 ± 27	231 ± 13	205 ± 29
BUN (mg/dL)	17.2 ± 2.7	16.6 ± 1.6	17.6 ± 1.6	16.4 ± 2.6
Creatinine (mg/dL)	0.50 ± 0.00	0.51 ± 0.04	0.51 ± 0.04	0.50 ± 0.05
Sodium (mmol/L)	147 ± 1	148 ± 1	148 ± 1	147 ± 1
Potassium (mmol/L)	5.7 ± 0.3	5.9 ± 0.7	6.1 ± 0.7	5.8 ± 0.6
Calcium (mg/dL)	11.6 ± 0.3	11.6 ± 0.4	11.6 ± 0.4	11.5 ± 0.4
Magnesium (mg/dL)	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.1	2.7 ± 0.2
Phosphorus (mg/dL)	8.3 ± 0.9	7.8 ± 0.8	8.8 ± 0.7	8.6 ± 0.6
Cholesterol (mg/dL)	62 ± 7 (n=7)	58 ± 8	69 ± 7	66 ± 11
Triglycerides (mg/dL)	120 ± 32	115 ± 31	154 ± 76	102 ± 38
Total Protein (g/dL)	6.3 ± 0.3	6.2 ± 0.2	6.3 ± 0.3	6.2 ± 0.3
Albumin (g/dL)	3.3 ± 0.2	3.2[b] ± 0.1	3.3 ± 0.1	3.2 ± 0.2
Globulin (g/dL)	3.0 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	3.0 ± 0.2
ALB/GLOB (%)	1.1 ± <0.1	1.1 ± <0.1	1.1 ± 0.0	1.1 ± 0.1
AST (IU/L)	94 ± 11	84 ± 11	99 ± 40	94 ± 13
ALT (IU/L)	46 ± 6	50 ± 5	54 ± 10	54 ± 8
ALKP (IU/L)	230 ± 62	206 ± 35	249 ± 55	224 ± 46

[a]Mean ± SD

[b]$p<0.05$ compared to control

TABLE 6. HEMATOLOGY VALUES[a] FOR MALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=8	n=8	n=8	n=8
WBC ($x10^3/mm^3$)	5.9 ± 1.1	6.2 ± 0.7	7.2 ± 1.2	7.6 ± 1.9
Neutrophils (%)	19 ± 3	19 ± 11	16 ± 4	19 ± 9
Lymphocytes (%)	77 ± 4	77 ± 14	79 ± 6	80 ± 7 (n=7)
Monocytes (%)	2.2 ± 2.8	1.6 ± 2.6	2.3 ± 3.9	0.9 ± 0.5 (n=7)
Esoinophils (%)	1.8 ± 0.4	2.2 ± 0.5	2.2 ± 1.0	2.0 ± 0.8
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RBC ($x10^6/mm^3$)	9.09 ± 0.43	8.81 ± 0.57	8.90 ± 0.27	8.77 ± 0.29
HGB (g/dL)	15.3 ± 0.5	15.3 ± 0.6	15.0 ± 0.4	15.1 ± 0.8
HCT (%)	49 ± 2	48 ± 2	48 ± 1	48 ± 2
MCV (fL)	54 ± 1	55 ± 3	54 ± 2	55 ± 2
MCH (pg)	17 ± <1	17 ± 1	17 ± 1	17 ± 1
MCHC (g/dL)	31 ± <1	32[b] ± <1	31 ± <1	32 ± 1
PLT ($x10^3/mm^3$)	1141 ± 155	1224 ± 119	1210 ± 158	1169 ± 172

[a]Mean ± SD
[b]$p<0.05$ compared to control

TABLE 7. SERUM CHEMISTRY VALUES[a] FOR MALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=8	n=8	n=8	n=8
Glucose (mg/dL)	202 ± 15	194 ± 21	193 ± 18	196 ± 24
BUN (mg/dL)	17.0 ± 1.3	18.1 ± 1.6	16.5 ± 1.4	17.6 ± 2.3
Creatinine (mg/dL)	0.81 ± 0.27	0.89 ± 0.23	0.91 ± 0.24	0.86 ± 0.28
Sodium (mmol/L)	149 ± 2	149 ± 1	149 ± 2	150 ± 1
Potassium (mmol/L)	6.2 ± 1.2	5.9 ± 0.8	5.8 ± 0.8	5.6 ± 0.7
Calcium (mg/dL)	11.7 ± 0.3	11.5 ± 0.4	11.6 ± 0.3	11.6 ± 0.2
Magnesium (mg/dL)	3.2 ± 0.1	3.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.1
Phosphorus (mg/dL)	7.9 ± 0.6	8.1 ± 1.1	8.2 ± 0.7	7.9 ± 0.6
Cholesterol (mg/dL)	71 ± 11	67 ± 13 (n=7)	84 ± 15	77 ± 15
Triglycerides (mg/dL)	133 ± 21	154 ± 63	168 ± 61	135 ± 46
Total Protein (g/dL)	7.1 ± 0.2	6.7[b] ± 0.3	7.0 ± 0.1	6.9 ± 0.2
Albumin (g/dL)	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	4.1 ± 1.3
Globulin (g/dL)	3.4 ± 0.1	3.2[b] ± 0.1	3.3 ± 0.1	3.8 ± 1.5
ALB/GLOB (%)	1.1 ± <0.1	1.1 ± <0.1	1.1 ± <0.1	1.1 ± <0.1
AST (IU/L)	252 ± 133	302 ± 117	321 ± 123	311 ± 133
ALT (IU/L)	57 ± 4	59 ± 10	59 ± 12	62 ± 9
ALKP (IU/L)	205 ± 40	176 ± 50	204 ± 58	244 ± 56

[a]Mean ± SD

[b]$p<0.05$ compared to control

TABLE 8. HEMATOLOGY VALUES[a] FOR FEMALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=16	n=15	n=16	n=16
WBC ($x10^3/mm^3$)	5.1 ± 0.9	4.8 ± 0.8	4.6 ± 1.4	4.4[b] ± 0.9
Neutrophils (%)	13 ± 5	14 ± 5	12 ± 5	10 ± 6
Lymphocytes (%)	84 ± 5	82 ± 5	84 ± 6	87 ± 5
Monocytes (%)	1.5 ± 2.2	1.8 ± 2.7	2.4[b] ± 2.4	1.2 ± 0.7
Esoinophils (%)	1.9 ± 0.5	2.0 ± 0.9	2.0 ± 0.5	1.6 ± 0.6
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.1 ± 0.4
RBC ($x10^6/mm^3$)	7.99 ± 0.35	7.89 ± 0.41	7.87 ± 0.37	7.75 ± 0.36
HGB (g/dL)	14.7 ± 0.4	14.4 ± 0.8	14.5 ± 0.5	14.0[d] ± 0.6
HCT (%)	46 ± 1	45 ± 2	46 ± 2	44[d] ± 2
MCV (fL)	58 ± 1	58 ± 1	58 ± 2	57 ± 1
MCH (pg)	18 ± <1	18 ± <1	18 ± <1	18[b] ± 1
MCHC (g/dL)	32 ± <1	32 ± <1	32 ± <1	32 ± 1
PLT ($x10^3/mm^3$)	1025 ± 97	1062 ± 127	1053 ± 181	1096 ± 121

[a]Mean ± SD
[b]$p<0.05$ compared to control
[c]$p<0.01$ compared to control
[d]$p<0.001$ compared to control

TABLE 9. SERUM CHEMISTRY VALUES[a] FOR FEMALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
	n=16	n=15	n=16	n=16
Glucose (mg/dL)	169 ± 18	162 ± 16	159 ± 14	161 ± 15
BUN (mg/dL)	18.9 ± 2.1	19.4 ± 2.5	19.7 ± 2.5	16.8[b] ± 2.3
Creatinine (mg/dL)	0.66 ± 0.10	0.73 ± 0.16	0.66 ± 0.07	0.71 ± 0.17
Sodium (mmol/L)	150 ± 2	149 ± 2	150 ± 1	150 ± 3
Potassium (mmol/L)	5.8 ± 0.8	5.5 ± 0.6	5.7 ± 0.5	5.5 ± 0.7
Calcium (mg/dL)	11.9 ± 0.5	12.0 ± 0.5	12.0 ± 0.7	12.0 ± 0.4
Magnesium (mg/dL)	3.2 ± 0.3	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.2
Phosphorus (mg/dL)	7.7 ± 0.9	7.9 ± 0.8	8.0 ± 1.2	7.6 ± 0.8
Cholesterol (mg/dL)	73 ± 11	85 ± 21	94[d] ± 19	98[d] ± 14
Triglycerides (mg/dL)	136 ± 59	189 ± 108	184[b] ± 67	124 ± 50
Total Protein (g/dL)	7.3 ± 0.5	7.5 ± 0.5	7.8[c] ± 0.4	7.6[b] ± 0.4
Albumin (g/dL)	4.1 ± 0.4	4.3 ± 0.5	4.5[c] ± 0.4	4.4[b] ± 0.4
Globulin (g/dL)	3.2 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.2 ± 0.2
ALB/GLOB (%)	1.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	1.4[b] ± 0.1
AST (IU/L)	160 ± 62	196 ± 84	178 ± 55	184 ± 82
ALT (IU/L)	59 ± 13	86 ± 91	69 ± 28	53 ± 12
ALKP (IU/L)	164 ± 50	133 ± 47	130[b] ± 51	135 ± 52

[a]Mean ± SD
[b]$p<0.05$ compared to control
[c]$p<0.01$ compared to control
[d]$p<0.001$ compared to control

TABLE 10. SERUM THYROID HORMONE VALUES[a] FOR MALE RATS EXPOSED TO CF_3I FOR 7 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
TSH(ng/mL)	3.58 ± 0.30	5.50[d] ± 0.55	5.96[d] ± 0.42	5.94[d] ± 0.54
T_4 (ug/dL)	3.46 ± 0.31	5.48[d] ± 0.51	7.56[d] ± 0.80	8.74[d] ± 0.82
T_3 (ng/dL)	138 ± 11	121[b] ± 12	113[d] ± 8	108[d] ± 9
rT_3 (ng/dL)	4.76 ± 0.45	6.79[d] ± 0.49	7.56[d] ± 0.73	14.5[d] ± 1.1

[a]Mean ± SD, n=7 to 9.
[b]$p<0.05$ compared to control
[c]$p<0.01$ compared to control
[d]$p<0.001$ compared to control

TABLE 11. SERUM THYROID HORMONE VALUES[a] FOR MALE AND FEMALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	Sex	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
TSH(ng/mL)	M	3.77 ± 0.33	3.66 ± 0.40	3.87 ± 0.40	6.60[d] ± 0.68
	F	6.60 ± 0.75	7.99[b] ± 0.82	7.61 ± 0.81	17.96[d] ± 1.91
T_4 (ug/dL)	M	3.80 ± 0.35	5.32[d] ± 0.56	7.28[d] ± 0.76	7.80[d] ± 0.56
	F	4.09 ± 0.46	7.50[d] ± 0.78	8.91[d] ± 1.00	10.43[d] ± 1.06
T_3 (ng/dL)	M	143 ± 8	107[d] ± 12	115[d] ± 10	105[d] ± 10
	F	169 ± 17	145[d] ± 16	125[d] ± 15	107[d] ± 11
rT_3 (ng/dL)	M	4.55 ± 0.53	7.43[d] ± 0.79	9.03[d] ± 0.90	13.1[d] ± 1.28
	F	6.40 ± 0.70	11.1[d] ± 1.14	14.4[d] ± 1.30	25.7[d] ± 2.23

[a]Mean ± SD, n=15 or 16 for females, 8 for males
[b]$p<0.05$ compared to control
[c]$p<0.01$ compared to control
[d]$p<0.001$ compared to control

TABLE 12. MICRONUCLEI SCORES[a] FOR MALE AND FEMALE RATS EXPOSED TO CF_3I FOR 7 OR 14 WEEKS

Time	Sex	n	Endpoint	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
7 wk	M	8	PCE/NCE[b]	0.29 ± 0.19	0.26 ± 0.13	0.43 ± 0.17	0.40 ± 0.15
7 wk	M	8	% MN[c]	0.34 ± 0.17	0.29 ± 0.16	0.46 ± 0.16	0.37 ± 0.21
14 wk	M	8	PCE/NCE	0.29 ± 0.12	0.32 ± 0.17	0.26 ± 0.08	0.27 ± 0.13
14 wk	M	8	% MN	0.64 ± 0.60	0.43 ± 0.26	0.41 ± 0.33	0.37 ± 0.31
14 wk	F	16	PCE/NCE	0.30 ± 0.19	0.26[d] ± 0.19	0.29 ± 0.19	0.25 ± 0.11
14 wk	F	16	% MN	0.45 ± 0.28	0.50[d] ± 0.42	0.42 ± 0.24	0.66 ± 0.43

[a]Mean ± SD
[b]Polychromatic erythrocytes/normochromatic erythrocytes
[c]Percentage of micronucleated cells/polychromatic erythrocytes
[d]n=15

Reproductive Data

Results of the reproductive assessment are given in Table 13. There were no statistically significant differences between CF_3I-treated and control groups in all indices and endpoints measured, except for a low pup sex ratio (males:females) in the 2.0% group.

Gross Necropsy and Organ Weights

There were no clinically significant or CF_3I-exposure related gross lesions in any of the study animals. The Pathology Report is given in Appendix B. Mean absolute and relative (to body weight) organ weights of male rats exposed to CF_3I for 7 or 14 wk are given in Tables 14 and 15, respectively. Mean absolute and relative organ weight values for female rats are given in Table 16. Statistically significant differences from the organ weight means of the control animals follow. Relative liver weights were mildly increased (6-10% above control values) in the male rats of the 2.0% group at both the 7-wk and 14-wk sacrifice periods. Mean absolute and relative epididymides weights were also increased in male rats of the 0.7 and 2.0% groups at 7 wk, but not at 14 wk. The increase in epididymides weights was not CF_3I-concentration dependent. For female rats, decreases in mean absolute organ weights included the brain (0.7 and 2.0% CF_3I), ovaries (0.7 and 2.0%), and heart (2.0%). Mean relative brain (2.0%), kidney (2.0%), and liver (0.7%) weights were increased. Mean relative ovaries weights were decreased (0.7 and 2.0% CF_3I). Note that mean body weight was statistically significantly decreased in the 2.0% female rats compared to the control female rats (Table 16).

TABLE 13. REPRODUCTIVE DATA FOR RATS EXPOSED TO CF_3I FOR 14 WEEKS

Parameter	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
No. of Females Paired (placed with a male)	16	16	16	16
Mating Index[a] (%)	100	94	94	81
Fecundity Index[b] (%)	94	80	100	85
Fertility Index[c] (%)	94	75	94	69
Mean[d] Gestation Length (days)	22.2 ± 0.7	22.4 ± 0.5	22.2 ± 0.4	22.6 ± 0.5
Gestation Index (%)[e]	100	100	100	91
Mean[d] No. of Pups per Litter	13.3 ± 4.2	12.5 ± 4.6	14.7 ± 2.3	11.6 ± 3.9
Pup Sex Ratio[f]	0.99	0.79	1.07	0.68[k]
Live Birth Index[d,g] (%)	100 ± 0	99 ± 5	99 ± 3	90 ± 30
4-Day Survival Index[d,h] (%)	99 ± 2	100 ± 0	98 ± 3	98 ± 3
7-Day Survival Index[d,j] (%)	100 ± 0	97 ± 11	98 ± 4	100 ± 0
14-Day Survival Index[d,j] (%)	100 ± 0	97 ± 11	98 ± 4	100 ± 0
21-Day Survival Index[d,j] (%)	100 ± 0	97 ± 11	98 ± 4	100 ± 0

[a] $\frac{\text{Number of females with plug or sperm positive} \times 100}{\text{Number of females paired}}$

[b] $\frac{\text{Number of females delivering a litter} \times 100}{\text{Number of females with plug or sperm positive}}$

[c] $\frac{\text{Number of females delivering a litter} \times 100}{\text{Number of females paired}}$

[d] Mean ± SD

[e] $\frac{\text{Number of females with live litters} \times 100}{\text{Number of females delivering a litter}}$

[f] $\frac{\text{Number of male pups per group}}{\text{Number of female pups per group}}$

[g] $\frac{\text{Number of live pups at birth} \times 100}{\text{Number of pups born}}$

[h] $\frac{\text{Number of pups surviving 4 days} \times 100}{\text{Number of live pups at birth}}$

[j] $\frac{\text{Number of pups surviving 7-, 14-, or 21 days} \times 100}{\text{Number of pups retained at 4 days}}$

[k] $p<0.05$ compared to control

TABLE 14. ABSOLUTE AND RELATIVE ORGAN WEIGHTS[a] OF MALE RATS EXPOSED TO CF_3I FOR 7 WEEKS

Organ	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
Body weight	516 ± 26	504 ± 27	527 ± 36	499 ± 53
Brain	2.08 ± 0.09	2.07 ± 0.09	2.11 ± 0.10	2.10 ± 0.10
Ratio[b]	0.40 ± 0.03	0.41 ± 0.03	0.40 ± 0.02	0.42 ± 0.04
Liver	18.60 ± 2.74	18.22 ± 1.90	19.62 ± 2.54	19.90 ± 2.91
Ratio	3.59 ± 0.39	3.61 ± 0.29	3.71 ± 0.27	3.98[e] ± 0.25
Kidneys	3.50 ± 0.28	3.37 ± 0.34	3.45 ± 0.55	3.60 ± 0.39
Ratio	0.68 ± 0.04	0.67 ± 0.06	0.65 ± 0.07	0.72 ± 0.05
Testes	3.26 ± 0.24	3.28 ± 0.23	3.46 ± 0.31	3.43 ± 0.22
Ratio	0.63 ± 0.06	0.65 ± 0.06	0.66 ± 0.05	0.69 ± 0.06
Spleen	0.82 ± 0.14	0.79 ± 0.11	0.84 ± 0.15	0.84 ± 0.15
Ratio	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.17 ± 0.02
Thymus	0.42 ± 0.13	0.45 ± 0.08	0.48 ± 0.15	0.39 ± 0.11
Ratio	0.08 ± 0.02	0.09 ± 0.01	0.09 ± 0.03	0.08 ± 0.02
Heart	1.48 ± 0.11	1.51 ± 0.08	1.64 ± 0.26	1.54 ± 0.17
Ratio	0.29 ± 0.01	0.30 ± 0.01	0.31 ± 0.04	0.31 ± 0.02
Epididymides	1.26 ± 0.11	1.33 ± 0.11	1.56[e] ± 0.24	1.46[e] ± 0.20
Ratio	0.25 ± 0.03	0.27 ± 0.04	0.29[f] ± 0.03	0.29[e] ± 0.05
Adrenal Glands	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Ratio	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lungs	2.27 ± 0.13	2.29 ± 0.25	2.24 ± 0.25	2.19 ± 0.19
Ratio	0.44 ± 0.02	0.45 ± 0.04	0.42 ± 0.03	0.44 ± 0.03
Thyroid[c]	20 ± 5	21[d] ± 3	21 ± 3	24 ± 5
Ratio[c]	3.9 ± 0.8	4.2[d] ± 0.5	3.9 ± 0.6	4.8 ± 1.0

[a]Mean ± SD, n=8, grams
[b]Organ weight/body weight x100
[c]mg (absolute) or mg/g x 100 (ratio)
[d]n=7
[e]$p<0.05$ compared to control
[f]$p<0.01$ compared to control

TABLE 15. ABSOLUTE AND RELATIVE ORGAN WEIGHTS[a] OF MALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Organ	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
Body weight	595 ± 26	599 ± 79	586 ± 51	567 ± 74
Brain	2.16 ± 0.14	2.15 ± 0.10	2.16 ± 0.13	2.20 ± 0.09
Ratio[b]	0.36 ± 0.03	0.36 ± 0.04	0.37 ± 0.02	0.40 ± 0.06
Liver	20.20 ± 1.42	19.72 ± 3.52	20.06 ± 2.67	20.41[d] ± 3.25
Ratio	3.40 ± 0.15	3.28 ± 0.23	3.41 ± 0.19	3.60[d,e] ± 0.17
Kidneys	3.62 ± 0.31	3.60 ± 0.48	3.63 ± 0.36	3.74 ± 0.42
Ratio	0.61 ± 0.05	0.60 ± 0.04	0.62 ± 0.02	0.66 ± 0.05
Testes	3.43 ± 0.15	3.48 ± 0.27	3.53 ± 0.20	3.30[d] ± 0.18
Ratio	0.58 ± 0.03	0.59 ± 0.09	0.61 ± 0.06	0.60[d] ± 0.11
Spleen	0.79 ± 0.06	0.79 ± 0.07	0.78 ± 0.06	0.87 ± 0.13
Ratio	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.15 ± 0.02
Thymus	0.39 ± 0.12	0.37 ± 0.05	0.41 ± 0.09	0.38 ± 0.12
Ratio	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Heart	1.63 ± 0.14	1.69 ± 0.17	1.70 ± 0.18	1.73 ± 0.23
Ratio	0.27 ± 0.02	0.28 ± 0.02	0.29 ± 0.04	0.31 ± 0.04
Epididymides	1.48 ± 0.12	1.61 ± 0.36	1.54 ± 0.13	1.49 ± 0.14
Ratio	0.25 ± 0.02	0.27 ± 0.06	0.26 ± 0.03	0.27 ± 0.03
Adrenal Glands	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Ratio	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Lungs	2.54 ± 0.22	2.37 ± 0.22	2.42 ±0.19	2.51[d] ± 0.45
Ratio	0.43 ± 0.03	0.40 ± 0.03	0.41 ± 0.03	0.44[d] ± 0.03
Thyroid[c]	23 ± 2	24 ± 3	22 ± 3	22 ± 3
Ratio[c]	3.9 ± 0.3	4.0 ± 0.6	3.8 ± 0.7	4.0 ± 0.6

[a]Mean ± SD, n=8, grams
[b]Organ weight/body weight x100
[c]mg (absolute) or mg/g x 100 (ratio)
[d]n=7
[e]$p<0.05$ compared to control

TABLE 16. ABSOLUTE AND RELATIVE ORGAN WEIGHTS[a] OF FEMALE RATS EXPOSED TO CF_3I FOR 14 WEEKS

Organ	0.0% CF_3I	0.2% CF_3I[d]	0.7% CF_3I	2.0% CF_3I
Body weight	321 ± 27	322 ± 25	314 ± 19	289[f] ± 36
Brain	1.96 ± 0.05	1.92 ± 0.12	1.89[e] ± 0.10	1.87[g] ± 0.07
Ratio[b]	0.61 ± 0.04	0.60 ± 0.05	0.61 ± 0.06	0.66[e] ± 0.08
Liver	11.09 ± 1.51	11.67 ± 1.18	11.80 ± 1.45	10.54 ± 1.83
Ratio	3.45 ± 0.31	3.62 ± 0.29	3.76[e] ± 0.30	3.64 ± 0.34
Kidneys	2.05 ± 0.15	2.10 ± 0.27	2.03 ± 0.13	1.98 ± 0.22
Ratio	0.64 ± 0.04	0.65 ± 0.10	0.65 ± 0.04	0.69[f] ± 0.04
Ovaries	0.13 ± 0.01	0.12 ± 0.03	0.12[e] ± 0.03	0.10[g] ± 0.02
Ratio	0.04 ± 0.01	0.04 ± 0.01	0.04[e] ± 0.01	0.03[g] ± 0.00
Spleen	0.54 ± 0.06	0.55 ± 0.10	0.53 ± 0.09	0.48 ± 0.09
Ratio	0.17 ± 0.01	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.02
Thymus	0.33 ± 0.06	0.32 ± 0.08	0.29 ± 0.09	0.28 ±0.07
Ratio	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.02
Heart	1.13 ± 0.11	1.09 ± 0.10	1.11 ± 0.09	1.01[e] ± 0.13
Ratio	0.35 ± 0.04	0.34 ± 0.03	0.35 ± 0.02	0.35 ± 0.02
Adrenal Glands	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.01
Ratio	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Lungs	1.80 ± 0.14	1.72 ± 0.13	1.73 ± 0.18	1.68 ± 0.18
Ratio	0.56 ± 0.05	0.54 ± 0.05	0.55 ± 0.05	0.59 ± 0.04
Thyroid[c]	18 ± 3	19 ± 2	19 ± 3	19 ± 4
Ratio[c]	5.5 ± 1.0	5.8 ± 0.7	6.0 ± 1.1	6.6 ± 1.6

[a]Mean ± SD, n=16, grams
[b]Organ weight/body weight x100
[c]mg (absolute) or mg/g x 100 (ratio)
[d]n=15, except absolute and relative thyroid values where n=14
[e]$p<0.05$ compared to control
[f]$p<0.01$ compared to control
[g]$p<0.001$ compared to control

Histopathology

Details of the tissue histopathologic findings are given in the Pathology Report, Appendix B. There were no lesions of clinical significance in any CF_3I-treated (2.0% group) or control group animals. Tissues from animals in the 0.7 or 0.2% CF_3I groups were not examined microscopically, due to the lack of histopathologic findings in rats of the control and 2.0% CF_3I groups.

Progeny

Clinical Observations and Pup Weights

There were no CF_3I-treatment related clinical observations in the first generation pups from birth to postnatal Day 21. Pup survival indices are given in Table 13. There were no statistically significant differences between control and CF_3I exposure groups in mean pup survival indices. The means of male pup weights are given in Table 17. Means of female pup weights are given in Table 18. There were no statistically significant differences between control and CF_3I exposure groups in mean pup weights.

TABLE 17. MALE PUP WEIGHTS[a]

Postnatal Day	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
0	6.87 ± 0.74 (15)	7.40 ± 0.71 (11)	6.92 ± 0.74 (15)	7.53 ± 0.76 (10)
4	9.64 ± 1.70 (15)	10.67 ± 1.44 (11)	9.78 ± 1.32 (15)	10.92 ± 1.64 (10)
7	15.30 ± 2.19 (15)	16.01 ± 1.66 (11)	15.62 ± 1.74 (15)	15.77 ± 2.02 (10)
14	28.94 ± 2.97 (15)	29.27 ± 2.20 (10)	28.64 ± 2.58 (15)	28.38 ± 2.24 (10)
21	48.45 ± 6.51 (15)	50.03 ± 4.99 (10)	47.34 ± 4.12 (15)	46.91 ± 5.30 (10)

[a]Mean ± SD, grams, (n)

TABLE 18. FEMALE PUP WEIGHTS[a]

Postnatal Day	0.0% CF_3I	0.2% CF_3I	0.7% CF_3I	2.0% CF_3I
0	6.56 ± 0.76 (15)	6.98 ± 0.72 (12)	6.43 ± 0.65 (15)	6.98 ± 0.64 (10)
4	9.34 ± 1.74 (15)	10.09 ± 1.60 (12)	9.13 ± 1.30 (15)	10.07 ± 1.41 (10)
7	14.59 ± 2.27 (15)	15.34 ± 1.39 (12)	14.64 ± 1.72 (15)	14.31 ± 1.85 (10)
14	27.67 ± 3.03 (15)	28.02 ± 2.35 (11)	27.16 ± 2.37 (15)	26.34 ± 2.24 (10)
21	46.00 ± 6.27 (15)	47.79 ± 4.65 (11)	44.86 ± 4.29 (15)	43.68 ± 4.42 (10)

[a]Mean ± SD, grams, (n)

SECTION IV

DISCUSSION

This study met its objective, that is, to determine and evaluate the potential of CF_3I to produce alterations in parental fertility; maternal pregnancy and lactation; and growth and development of offspring in the rat. A no-observable-effect-level (NOEL) is recommended (below). Additionally, questions raised on the effects of CF_3I exposure following the recently completed acute and subchronic inhalation toxicity studies (Dodd *et al.*, 1997a) were answered.

A brief synopsis of the results of this study and interpretation, where necessary, follows. In parental animals, there were no clinical signs of toxicity. A minimal decrease in mean body weight in rats of the 2.0% CF_3I group was observed, though the decrease was not statistically significant, except at the conclusion of the study (female rats only). Statistically significant differences in hematology and serum chemistry parameters (except thyroid hormones) were few in number and not considered treatment related or biologically important due to 1) lack of consistency with time (7-wk compared to 14-wk values), 2) lack of dose-response, or 3) the difference was small (clinically insignificant). Statistically significant, concentration-related increases in serum TSH, T_4, and rT_3 were observed. T_3 levels were decreased. The alterations in serum thyroid hormones caused by CF_3I exposure were similar to those observed previously in this laboratory (Dodd *et al.*, 1997a), and provide further support for the mechanistic theory of inhibition of 5'-deiodinase, an enzyme that catalyzes the conversion of T_4 to T_3. Reduced levels of T_3 initiates an increase in TSH. Increases in TSH lead to increases in T_4. When the conversion of T_4 to T_3 is inhibited, increases in rT_3 occur. Though there are toxicity concerns, such as thyroid goiter and tumor development, for chemicals that induce a sustained increase in the secretion of pituitary TSH, rats and mice are highly sensitive to these effects compared to humans (Capen, 1995; McClain *et al.*, 1988, 1989). Humans can have markedly altered changes in thyroid function and elevated TSH levels, as in areas with a high incidence of endemic goiter because of iodine deficiency, but there is little, if any, increase in the incidence of thyroid cancer (Curren and DeGroot, 1991). Thresholds for a "no-effect" level on the thyroid gland can be established by determining the dose of xenobiotic that fails to elicit an elevation in the circulating level of TSH (Capen, 1996, personal communication). In rodent studies, this "no effect" level would provide a wide margin of safety for danger to the human thyroid due to considerable differences in thyroid hormone economy and response of follicular cells to TSH between rodents and man (Capen, 1995, 1996, personal communication).

In the current investigation, there was no increase in micronuclei frequency in the bone marrow erythrocytes of rats exposed to CF_3I. Though CF_3I was positive in the Ames and mouse bone marrow erythrocyte tests (Dodd *et al.*, 1997b), and induction of micronuclei was observed in rats exposed to CF_3I at concentrations ranging from 2 to 8% (Dodd *et al.*, 1997a), the effect observed previously at 2% CF_3I is not reproducible. Additionally, the PCE/NCE ratio, an indicator of bone marrow toxicity, was unaltered in the current study. In the previous study (Dodd *et al.*, 1997a), the PCE/NCE ratio decreased indicating toxicity by CF_3I exposure. The difference in results of micronuclei scores and PCE/NCE ratios between the previous and current studies

suggests that stress, due to nose-only inhalation exposure confinement (previous study) may be a factor. Strain difference (Fisher 344 vs. Sprague Dawley CD) might also be a factor. Also, it is general knowledge that the dose of a test agent varies in animals when different inhalation exposure delivery systems (nose-only vs. whole-body) are used, though the exposure concentrations for each delivery system are the same. Nose-only systems generally involve higher rates of respiration because of the added stress of this route of exposure as compared with whole-body. This would result in a higher accumulative dose for animals exposed in a nose-only system.

In the current investigation, there were no treatment-related gross lesions at necropsy. Statistically significant differences in mean organ weights were few in number and not considered biologically important for the same reasons as cited above. CF_3I exposure did not cause microscopic changes in tissues and organs, including the target organ, the thyroid. Analysis of reproductive indices and parameters, including pup survival and growth, indicate CF_3I is not a reproductive toxicant. This analysis included statistical analyses of the current reproductive data and comparison of historical reproductive indices in control rats in five reproductive toxicity screens conducted at this laboratory from 1991-1996. Testicular lesions observed in rats exposed to 4 or 8% CF_3I (Dodd *et al.*, 1997a) may have been a result of heat stress, due to animals being placed in restraining tubes during the inhalation exposure regimen.

In conclusion, exposure of 2.0% CF_3I vapor for approximately 14 weeks produced minimal general toxicity and no reproductive toxicity in Sprague-Dawley rats. On the basis of general toxicity, reproductive toxicity, and serum TSH concentrations in the current study and in previous studies on CF_3I (Dodd *et al.*, 1997a, 1997b), the no-observable-effect-level (NOEL) is 0.7% CF_3I.

SECTION V

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QUALITY ASSURANCE

The study, "Reproductive Toxicity Screen of Trifluoroiodomethane (CF_3I) in Sprague-Dawley Rats," was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research under the guidance of the Environmental Protection Agency's Good Laboratory Practices Standards, 40 CFR 792.

The various phases of this study were inspected by members of the Quality Assurance Unit. Results of the inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION	ITEM INSPECTED
April 28, 1997	Animal Randomization
April 28, 1997	Animal body weights
April 28, 1997	Initiate inhalation exposure
May 19, 1997	Animal body weights
May 27, 1997	Mating period activities
June 2, 1997	Gestation period activities
June 2, 1997	Male rat body weights
June 2, 1997	Exposure system data
June 11, 1997	IP injection male rats for micronucleus samples
June 11, 1997	Terminal body weights, sacrifice male rats, collect micronuclei samples
July 14, 1997	Animal body weights
July 28, 1997	Terminal body weights, sacrifice male rats, collect bone marrow samples
July 29, 1997	IP injection female rats for bone marrow samples
July 30, 1997	Exposure system data
July 30, 1997	Terminal body weights, sacrifice female rats, collect bone marrow samples
January 20-29, 1998	Data and Final Report Audit
February 4, 1998	Review missing data and archive

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

M. G. Schneider
QA Coordinator
Toxic Hazards Research

Date February 11, 1998

APPENDIX A

CHEMICAL AND CHAMBER ATMOSPHERE ANALYSES

FOR THE 14-WEEK REPRODUCTION VAPOR INHALATION STUDY OF CF_3I

IN SPRAGUE-DAWLEY RATS

prepared by H. F. Leahy

(10 pages)

Chemical and Chamber Atmospheric Analysis for the 14-Week Reproduction Inhalation Study of Trifluoroiodomethane (CF_3I) in Sprague-Dawley Rats

SUMMARY

The concentration of CF_3I in the exposure chambers were monitored throughout the exposure by Absorption Infrared Spectroscopy (IR). The concentration was monitored on a twenty minute cycle with digital readings taken ten minutes into the chamber atmosphere portion of the cycle and four minutes into the five minute input air cycle. The overall means (standard deviation) were 2.02% (0.03), 0.71% (0.01), 0.20% (0.005) for target concentrations of 2.0%, 0.7% and 0.2% respectively. CF_3I was not detected in the control chamber.

CHEMICAL

Four 350 pound cylinders of CF_3I test material (CAS Number 2314-97-8, three of Lot # 071796-FC, one of Lot # 061398-FC) were received from Ajay North America, LLC. (Powder Springs Ga.) on Feb.14, Apr. 21, May 16 and May 27, 1997, respectively. They were assigned numbers1 through 4 as received. The chemical and physical properties of the material are described in Table A-1 and Table A-6. The purity was verified by GC/MS analysis when introducing each new cylinder into the generation system. All cylinders contained >99.7% CF_3I when tested immediately prior to their use, independent of time of cylinder storage. Analytical results from cylinder #3, when the CF_3I test material was nearly depleted, indicated no significant compositional change. Weekly checks for purity also indicated no significant compositional change of the test material.

EQUIPMENT

A Miran 1A infra-red spectrometer was used in conjunction with a 10 cm gas cell (Foxboro Analytical, South Norwalk, Ct.) and a Cole Palmer 8386 strip chart recorder (Cole Palmer, Chicago, Il) to monitor the concentration of each exposure chamber. The operating parameters for the three exposure chamber Mirans are presented in Table A-2a. A Miran 980 with a long path cell monitored the control chamber and laboratory (Table A-2b). The GC/MS data were obtained with a combination of Tekmar and Hewlett Packard equipment (Table A-3). Daily statistics were obtained using Excel for Windows Ver. 7.

The RH and temperature were monitored using the Hy-Cal combination RH and Temperature probes (Hy-Cal Engineering, El Monte, Ca. The Oxygen concentration of the high chamber was analyzed using a Hudson oxygen sensor Model 82T (MDA, Lincolnshire, Il.)

Chamber input air and test material flows were monitored and controlled using rotometers (Matheson, Horsham Pa. and Dwyer Instrument Co., Michigan In.) and pressures by Dwyer magnehelics. The rotometers were calibrated using the Gilibrator System (Gillian Instrument Corp., N.J.) or a Precision Wet Test Meter (CGA/Precision Scientific, Chicago Il.) The analytical cycling was controlled by Greylab 625 timers (Dimco-Grey, Centerville, Oh.)

CALIBRATION

Calibration of the Miran 1As used for chamber analysis was achieved by introduction of a series of standards bracketing the target concentrations following determination of the absorption maximum at the 9.2 to 9.3 micron band for each instrument. Calibration curves were determined from the absorption data using the Excel spreadsheet binomial fit. Absorbance at target concentration for each exposure chamber was checked weekly. A recalibration was performed if the drift was significant. The three main absorbance bands (9.3, 8.4 and 9.75 um) were monitored by the Miran 980 for the control chamber/room analyzer system in order to detect the difference at low concentrations between drift and CF_3I. No test compound was observed in the chamber, but on one occasion the room monitor observed an estimated 5ppm concentration due to a leak from the valve stem of the fourth tank.

CHAMBER ATMOSPHERE ANALYSIS

The exposure chambers were monitored by infrared absorbance from before the introduction of test material until the animals were removed approximately thirty minutes after stopping input of test material. Introduction of CF_3I coincided with the start of the 20 minute analytical cycles, fifteen minutes on the chamber and five on the dilution air. There were 18 cycles in the six hour exposure day; the first was not included in the days mean, nor was the coast down period following termination of input of the test compound. 90 % of target concentration was achieved within the first fifteen minutes of exposure. The sample flow rate through the analytical cell was 700 mL/minute giving fourteen cell volume changes per minute.

The daily analytical mean concentrations (standard deviation) were 2.02% (0.03), 0.71% (0.01), 0.20% (0.005) for the target concentrations of 2.0%, 0.7% and 0.2% respectively. CF_3I was not detected in the control chamber. The nominal mean concentrations were 2.08% (0.09), 0.72% (0.03), and 0.24% (0.015) for the target concentrations of 2.0%, 0.70%, and 0.20%. The ratio between analytical/nominal concentrations as calculated from the rotometer settings for the air and CF_3I were 0.97, 0.98 and 0.83 for the 2%, 0.7%, and 0.2% target concentrations respectively. No CF_3I was observed in the control chamber. The daily means are presented in Table A-4, and a summary in Table A-5.

CHAMBER OPERATION

The test exposures were conducted in the Toxic Hazard Research facility at Armstrong Laboratory, WPAFB, in the 760 L ambient chambers. Due to the expense of the test material the total chamber flow was limited to 70L/minute for the first 37 days giving six chamber

volume exchanges per hour. Following the reduction of the number of animals the flow was reduced to 50 L/minute for the remainder of the study giving five chamber volume exchanges per hour. The CF_3I was introduced into an 20 L/minute input air line at a 'T' connection. Further dilution to the final flow rate occurred at a bucking 'T' for further mixing before introduction into the chamber.

The chamber analytical sample was taken from a central location between the four cages. Previous to the start of the exposure a test was conducted to determine chamber compound distribution. Four positions were sampled in addition to the central location. Following a half hour run to reach stability at the normal location, samples were taken from inside of the cages at both the upper and lower level and both outer and inner cages. No difference in the tracing was observed except a spike on changing locations.

The means of the daily RH mean values (standard deviation) for the chambers were 54.8% (6.5), 50.5% (5.0), 53.4% (5.2) and 54.8% (6.5) for the 2%, 0.7%, 0.2% and control chambers respectively. The mean of the daily temperature mean values were 73.5 °F (1.6), 74.1 °F (1.7), 73.9 °F (1.8) and 74.2 °F (1.6) for the 2%, 0.7%, 0.2% and control chambers respectively. The chambers were maintained at a negative pressure of 0.3 inches of water by regulating the exhaust flow. The oxygen concentration of the 2% chamber was monitored on occasion and found to be approximately 19%.

GC/MS Analysis

Samples from the four cylinders were tested for purity by the combination GC/MS system and results compared to library mass spectra of CF_3I for specific compound identification. The results determined that the samples taken at the start of cylinder use were >99.78%, >99.97%, >99.98%a, and 100% and the sample of the third cylinder at end of use was essentially unchanged at >99.8%. These five analytical values are within the normal range of instrument and laboratory variability. This demonstrated that during the four-month period of storage and use, there was no decomposition in the CF_3I test material.

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Table A-1

Chemical and Physical Properties of Trifluoroiodomethane

Common Name:	Iodotrifluoromethane
Synonyms:	Trifluoromethyl Iodide
CAS Number:	2314-97-8
Formula:	CF3I
Molecular Weight:	195.91
Boiling Point:	-22.5 C
Specific Gravity:	2.096 @ 25 C
Vapor Pressure:	63.70 psi @ 25 C
Solubility (water):	Insoluble
Appearance/Odor:	Colorless Gas/ Sweet-sharp
Flash Point:	Non-flammable

Table A-2a

Miran 1A Operational Parameters

	Chamber 1	Chamber 2	Chamber 3
Miran 1A	No. 9244	No. 10870	No. 5788
Wavelength	9.15 um	9.45 um	9.25 um
Pathlength	10 cm	10 cm	10 cm
Range	0 - 1 Abs	0 - 1 Abs	0 - 1 Abs
Slit	2 mm	2 mm	2 mm
Zero Course	1X	1 X	1 X
Zero Fine (initial)	868	690	602
Meter Response	4	4	4

Table A-2b

Miran 980 Operational Parameters

Chamber 4 and Room Monitor

Miran 980	No. 11114
Wavelengths	8.4, 9.3, 9.75 um
Pathlength	0.75m
Range	Absorbance

Table A-3

GC/MS Operational Equipment/Parameters

Tekmar Cryofocusing Module, Serial No. 92176014

Tekmar 7000 Headspace Analyzer, Serial No. 92063014

Hewlett-Packard 5970B Series Mass Selective Detector, Serial No.2623A01335

Hewlett-Packard 5890A Gas Chromatograph, Serial No. 2623A07142

Hewlett-Packard Vectra 386/25 Data System. Serial No. 312A091695

Analytical Column: Chrompack Poraplot Q, 30 m X 0.32mm, 10um film thickness

GC program: 45 C - 10.0 min. / 12 C per min. / 175 C -5 min.

Table A-4

Summary Table for the Daily Mean Concentration of CF_3I in the Exposure Chambers

CF_3I Date	Chamber 1 % Nominal	Chamber 1 % Analyzed	Chamber 2 % Nominal	Chamber 2 % Analyzed	Chamber 3 % Nominal	Chamber 3 % Analyzed
28-Apr	1.88	2.07	0.72	0.71	0.21	0.21
29-Apr	1.86	1.93	0.68	0.71	0.21	0.21
30-Apr	2.00	2.03	0.74	0.70	0.22	0.19
1-May	2.01	2.04	0.73	0.72	0.22	0.20
2-May	1.96	2.01	0.68	0.72	0.22	0.20
5-May	1.91	1.99	0.64	0.72	0.21	0.20
6-May	1.89	2.04	0.63	0.72	0.21	0.20
7-May	2.04	2.06	0.66	0.71	0.21	0.20
8-May	2.10	1.99	0.70	0.70	0.21	0.20
9-May	2.10	2.01	0.70	0.71	0.21	0.21
12-May	2.11	2.02	0.71	0.70	0.21	0.20
13-May	2.11	2.01	0.70	0.70	0.21	0.20
14-May	2.06	1.97	0.69	0.71	0.21	0.20
15-May	2.10	1.98	0.71	0.74	0.23	0.22
16-May	2.05	2.00	0.72	0.72	0.23	0.20
19-May	2.10	1.98	0.71	0.71	0.24	0.19
20-May	2.13	2.00	0.72	0.72	0.24	0.20
21-May	2.12	2.02	0.70	0.71	0.24	0.20
22-May	2.10	2.00	0.70	0.71	0.23	0.20
23-May	2.11	2.03	0.71	0.71	0.24	0.20
26-May	2.19	1.99	0.70	0.71	0.24	0.20
27-May	2.15	2.01	0.70	0.70	0.24	0.20
28-May	2.13	2.01	0.71	0.70	0.24	0.20
29-May	2.13	2.05	0.70	0.70	0.24	0.19
30-May	2.07	2.00	0.71	0.71	0.24	0.20
31-May	2.34	2.00	0.75	0.70	0.26	0.20
1-Jun	2.33	2.00	0.73	0.70	0.25	0.20
2-Jun	2.16	1.98	0.70	0.71	0.23	0.20
3-Jun	2.11	1.98	0.70	0.70	0.23	0.20
4-Jun	2.17	2.01	0.72	0.70	0.24	0.20
5-Jun	2.22	2.00	0.72	0.70	0.23	0.20
6-Jun	2.11	2.00	0.70	0.69	0.23	0.20
7-Jun	2.06	1.98	0.70	0.70	0.22	0.19
8-Jun	2.04	1.98	0.70	0.70	0.22	0.19
9-Jun	2.05	1.99	0.71	0.69	0.22	0.20
10-Jun	2.05	2.02	0.71	0.70	0.22	0.20
11-Jun	2.06	2.02	0.69	0.70	0.22	0.20
12-Jun	2.08	2.04	0.73	0.70	0.24	0.19

13-Jun	2.03	2.04	0.73	0.70	0.25	0.19
14-Jun	2.02	2.01	0.72	0.70	0.24	0.19
15-Jun	2.01	2.00	0.71	0.70	0.23	0.20
16-Jun	2.03	2.03	0.71	0.70	0.24	0.19
17-Jun	2.01	2.02	0.71	0.70	0.24	0.19
18-Jun	2.00	2.02	0.72	0.69	0.24	0.20
19-Jun	1.99	2.04	0.73	0.70	0.24	0.20
20-Jun	1.96	2.05	0.74	0.70	0.24	0.20
21-Jun	2.00	1.98	0.72	0.70	0.24	0.19
22-Jun	2.00	1.99	0.72	0.70	0.24	0.20
23-Jun	2.02	2.07	0.73	0.70	0.24	0.19
24-Jun	2.00	2.04	0.72	0.70	0.24	0.19
25-Jun	2.03	2.07	0.73	0.71	0.25	0.20
26-Jun	1.98	2.05	0.74	0.71	0.24	0.20
27-Jun	1.98	2.09	0.74	0.70	0.25	0.20
28-Jun	1.97	2.04	0.73	0.70	0.25	0.19
29-Jun	1.98	2.02	0.72	0.70	0.24	0.20
30-Jun	1.97	2.06	0.74	0.71	0.25	0.20
1-Jul	1.98	2.05	0.73	0.70	0.25	0.20
2-Jul	2.02	2.04	0.73	0.70	0.25	0.20
3-Jul	2.06	2.05	0.71	0.70	0.24	0.20
4-Jul	2.14	1.99	0.76	0.70	0.25	0.19
5-Jul	2.23	2.01	0.80	0.70	0.26	0.20
6-Jul	2.23	2.01	0.79	0.70	0.26	0.20
7-Jul	2.23	2.04	0.79	0.70	0.26	0.20
8-Jul	2.23	2.06	0.78	0.71	0.26	0.20
9-Jul	2.21	2.04	0.78	0.71	0.26	0.20
10-Jul	2.16	2.05	0.76	0.71	0.26	0.20
11-Jul	2.11	2.05	0.75	0.71	0.26	0.20
12-Jul	2.03	2.02	0.73	0.71	0.25	0.20
13-Jul	2.01	2.01	0.73	0.70	0.25	0.20
14-Jul	2.08	2.05	0.74	0.72	0.26	0.21
15-Jul	2.08	2.05	0.74	0.71	0.26	0.20
16-Jul	2.11	2.06	0.74	0.71	0.26	0.21
17-Jul	2.12	2.04	0.74	0.71	0.26	0.20
18-Jul	2.11	2.05	0.73	0.70	0.25	0.20
21-Jul	2.11	2.03	0.74	0.72	0.25	0.20
22-Jul	2.09	2.04	0.74	0.71	0.25	0.20
23-Jul	2.11	2.05	0.75	0.70	0.25	0.20
24-Jul	2.11	2.04	0.73	0.69	0.25	0.20
25-Jul	2.10	2.08	0.75	0.71	0.25	0.21
28-Jul	2.05	2.05	0.75	0.72	0.25	0.20
29-Jul	2.14	2.04	0.74	0.70	0.25	0.21
30-Jul	2.11	2.03	0.74	0.71	0.25	0.21
31-Jul	2.12	2.07	0.74	0.71	0.25	0.21
Mean	**2.08**	**2.02**	**0.72**	**0.71**	**0.24**	**0.20**
Std Dev	**0.09**	**0.03**	**0.03**	**0.01**	**0.015**	**0.005**

Table A-5

Summary of Daily Values for Chamber Operation 28 April to 31 July 1997

		Temp (°F)	Relative Humidity (%)	% Conc. Nominal	% Conc. Analyzed
Chamber 1	Mean	73.5	54.8	2.08	2.02
	Std Dev	1.6	6.5	0.09	0.03
Chamber 2	Mean	74.1	50.5	0.72	0.71
	Std Dev	1.7	5.0	0.03	0.01
Chamber 3	Mean	73.9	53.4	0.24	0.20
	Std Dev	1.8	5.2	0.015	0.005
Chamber 4	Mean	74.2	54.8	0.0	0.0
	Std Dev	1.6	6.5		

Table A-6

DATA FROM THE CERTIFICATE OF ANALYSIS

Product: Iodotrifluoromethane Revision No.: 122695

Ajay North America, LLC.

Lot Number: 061396-FC

Analysis	Limits	Value
Purity Wt.% CF_3I:	>= 99.8%	99.9%
Acidity (as HI):	<= 1.0 ppm	< 1.0 ppm
Water:	<= 20 ppm	5 ppm
Nonvolatile Residue Wt.%	<= 0.01%	< 0.01%
Suspended Matter or Sediment:	None Visible	None Visible
Halogen Ion:	Pass Test	Pass Test

APPENDIX B

PATHOLOGY REPORT

FOR THE REPRODUCTIVE TOXICITY SCREEN OF CF_3I

IN SPRAGUE-DAWLEY RATS

prepared by J. H. English

(4 pages)

REPRODUCTIVE TOXICITY SCREEN OF TRIFLUOROIODOMETHANE (CF_3I) IN SPRAGUE DAWLEY RATS

Study number: 17-F
Start date: 20 Feb. 97

Principal Investigator: D.E. Dodd
Study Pathologist: J.H. English

NARRATIVE PATHOLOGY REPORT

The following represents a compilation of gross, histopathologic and clinical findings from the 14 week whole-body inhalation study on CF_3I. This study was designed to assess potential reproductive toxicity associated with inhalation of the compound, and to provide additional data to augment an earlier nose-only inhalation study with Fischer 344 rats, specifically:

1) To determine a No Adverse Effect Level (NOAEL) for the compound.
2) To determine whether testicular lesions noted in the earlier study were due to effects of the compound, or were a result of experimental design which involved placing the animals in restrictive inhalation chambers, thus increasing stress and body temperature. Testicular lesions noted in the earlier study were more severe at the 30 day interim necropsy than at the full 90 day study. The 30 day point in the study coincided with the replacement of exposure tubes with larger ones, and it was this fact that suggested that the seemingly contradictory findings in the two groups might be due to heat stress rather than chemical effect. Nevertheless, the males in the current study were divided into two groups (7 week and 14 week, 8 animals each) in order to fully explore the ramifications of the previous study findings.
3) To determine if genotoxic effects (positive bone marrow micronucleus assays) were present at lower dose levels.
4) To determine if thyrotoxic effects noted in the early study were present at lower dose levels, and if so, whether they represent a human health hazard.

GROSS OBSERVATIONS:

Occasional gross lesions were encountered at necropsy; however, there were no clinically significant or treatment related gross lesions noted in any of the exposed animals. One control female was found to have pyometra, which was subsequently determined to be due to infection with *Proteus mirabilis*. This was considered to be an incidental finding, most likely due to contamination during vaginal flushing during the mating protocol.

HISTOPATHOLOGY:

With the exception of mild cystic endometritis found in one control female (as noted in gross observations), there were no lesions of clinical significance in any control or high dose group animals. Due to the lack of findings in control (0%) and high dose (2.0%) groups, tissues in the low (0.2%) and medium (0.7%) dose groups were not examined histologically, in accordance with the study protocol.

CLINICAL PATHOLOGY:

1. Hematologic Parameters: There were no significant changes in hematology or routine serum chemistry in any test groups.
2. Thyroid Parameters:

- TSH levels were not significantly elevated in low (0.2%) or medium (0.7%) groups at 14 weeks; however, high (2.0%) dose males had almost a two fold increase in TSH , and females had almost a three fold increase in comparison to control groups.
- T_4 levels exhibited a dose related increase at all exposure concentrations, with over two fold increase at the high dose level in males and females.
- T_3 levels were significantly lower in exposed animals, in comparison to controls. Decreases were dose related in females; however, males in the 14 week group failed to show a steady dose related decrease. Males in the 7 week exposure did demonstrate a dose related decrease in T_3 levels however, and the results seen in the 14 week males may merely reflect the smaller size of the male groups, relative to the female group.
- rT_3 levels exhibited a dose related increase in all exposure concentrations, reaching an almost three fold increase in males and over four fold increase in females at the high dose level (2.0%).

DISCUSSION:

No significant gross or histologic lesions were noted in this study, which examined the effects of whole body inhalation of CF_3I at a high dose concentration of 2%. In an earlier study examining the effects of nose only inhalation exposure at 8%, 4% and 2% [1], we observed mild inflammation in the nasal turbinates at the medium and high dose levels, and moderate testicular degeneration in medium and high dose males at 90 days, with marked testicular degeneration in 100% of medium and high dose males at 30 days. Low dose 30-day males were not available for necropsy, due to a procedural problem which resulted in their deaths. In the current study, the complete lack of any testicular lesions lends credence to the theory that the earlier observed lesions were due to heat stress resulting from placement in the confining nose-only chambers. In any event, the current study demonstrated a NOAEL at the high dose level of 2.0%, for both testicular and nasal turbinate changes.

Significant clinical pathologic findings in this study were limited to alterations in thyroid hormone parameters. Serum TSH was elevated in the high dose group, and there were dose related increases in T_4 and $_rT_3$, and a dose related decrease in T_3. These alterations were similar to those seen in our previous study[1], and provide further support for the mechanistic theory of inhibition of 5'-deiodinase conversion of T_4 to T_3, resulting in inappropriate conversion of T_4 to the inactive rT_3, thus increasing rT_3 and decreasing levels of T_3. This results in a perceived hypothyroid status, with resulting increased pituitary secretion of TSH, and a corresponding increase in thyroid production of T_4. Capen[2] reviewed various mechanistic data for xenobiotic perturbations of the pituitary-thyroid axis, describing a similar mechanism for FD&C Red No. 3, a common food dye. Regardless of the underlying mechanism, the various goitrogenic xenobiotics share a

common result in that they cause a chronic elevation in serum TSH. Rodents are well known to be exquisitely sensitive to long term elevations in TSH, and in chronic studies will develop a continuum of thyroid proliferative lesions, progressing from hyperplasia to adenoma and carcinoma. Capen suggests that in rodents, a viable definition of a No Effect Level (NOEL) would consist of the dose level at which no elevation in serum TSH was detected. By this definition, a NOEL was seen in this study at the 0.7% exposure level.

CONCLUSION:

The goals of this study were achieved, and significant data were provided to assist the risk assessment process for human exposure to CF_3I. This study demonstrated no dose related reproductive abnormalities, gross or histopathologic lesions, or mutagenic alterations (bone marrow micronucleus assay) in male or female Sprague Dawley rats at the high exposure dose of 2.0%. Given the complete lack of testicular lesions in any animals in this study, it is likely that testicular degeneration noted in the previous nose-only exposure[1] was in fact induced by stress and elevated body temperatures from confinement in close-fitting exposure chambers.

Following the NOEL definition recommended by Capen[2], a NOEL for thyrotoxic effects (no elevation in serum TSH levels) was seen at 0.7% exposure concentration. TSH elevation was noted at the high dose level of 2.0%, and presumably chronic exposure at this level in rats would result in proliferative lesions in the thyroid gland; however, it is well known that there are profound species differences between human and rodents in thyroid physiology, and that humans do not share rodents' sensitivity to elevations in TSH. This is due in part to the shorter half life of T_4 in rodents (12-24 hours) compared to humans (5-9 days), and to the markedly higher affinity for T_4 of thyroxine binding globulin (TBG) in humans, compared to prealbumin in rodents[2]. Though increases in TSH were noted in the high dose group in this study, the increases are of interest only in rodents, and are extremely unlikely to pose any risk hazard to humans in similar exposure scenarios.

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REFERENCES:

1. Dodd, D.E.; Kinkead, E.R.; Wolfe, R.E.; Leahy, H.F.; English, J.H.; Vinegar, A: Acute and Subchronic Inhalation Studies on Trifluoroiodomethane Vapor in Fischer 344 Rats. Fundamental and Applied Toxicology 35,64-77 (1997).

2. Capen, C.C., Mechanistic Data and Risk Assessment of Selected Toxic End Points of the Thyroid Gland. Toxicologic Pathology ISSN:0192-6233, Vol 25, No 1, 1997.